

# Association of Low B Cell Count and IgG Levels With Infection, and Poor Vaccine Response With All-Cause Mortality in an Immunosuppressed Vasculitis Population

David Morgan, Matthew; Richter, Alex; Al-Ali, Samer; Flint, Julia; Yiannakis, Constantina; Drayson, Mark; Goldblatt, David; Harper, Lorraine

DOI:  
[10.1002/acr.22757](https://doi.org/10.1002/acr.22757)

License:  
None: All rights reserved

*Document Version*  
Peer reviewed version

*Citation for published version (Harvard):*  
David Morgan, M, Richter, A, Al-Ali, S, Flint, J, Yiannakis, C, Drayson, M, Goldblatt, D & Harper, L 2016, 'Association of Low B Cell Count and IgG Levels With Infection, and Poor Vaccine Response With All-Cause Mortality in an Immunosuppressed Vasculitis Population', *Arthritis Care & Research*, vol. 68, no. 6, pp. 853-60. <https://doi.org/10.1002/acr.22757>

[Link to publication on Research at Birmingham portal](#)

## **Publisher Rights Statement:**

This is the peer reviewed version of the following article: Morgan, Matthew David, et al. "Low B cells and IgG are associated with infection, while poor vaccine response predicts all-cause mortality in an immunosuppressed vasculitis population." *Arthritis care & research* (2015)., which has been published in final form at <http://dx.doi.org/10.1002/acr.22757>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving

Checked October 2015

## **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

**Low B cells and IgG are associated with infection, while poor vaccine response predicts all-cause mortality in an immunosuppressed vasculitis population**

Matthew David Morgan<sup>1</sup>, Alex Richter<sup>1</sup>, Samer Al-Ali<sup>1</sup>, Julia Flint<sup>1</sup>, Constantina Yiannakis<sup>1</sup>, Mark Drayson<sup>1</sup>, David Goldblatt<sup>2</sup>, Lorraine Harper<sup>1</sup>.

1 School of Immunity and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

2 Institute of Child Health, University College London, London, UK

Corresponding author:

Lorraine Harper

Affiliation: School of Immunity and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

Postal address: Centre for Translational Inflammation Research, University of Birmingham Research Laboratories, 1st Floor, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Edgbaston, Birmingham, UK, B15 2WB

Telephone: +44 (0) 121 371 3238

Fax: +44 (0) 0121 371 5858

email: L.harper@bham.ac.uk

**Disclosure**

Dr. Goldblatt reports grants from Vaccine manufacturers: GSK, Sanofi Pasteur, Merck, Novartis, personal fees from Vaccine manufacturers: GSK, Sanofi Pasteur, Merck, Novartis, Pfizer, outside the submitted work.

Dr Alex Richter reports grant funding from Pfizer outside the submitted work

None of the other authors has relevant potential conflicts of interest to disclose.

Abstract word count :262

Manuscript word count 2857

Running title vaccination and vasculitis

## **ABSTRACT**

### **Objectives**

**Patients with systemic vasculitis (SV) have an increased risk of all-cause mortality, often due to infection, compared to the healthy population. We investigated whether humoral response to vaccination and biomarkers of immune dysfunction were associated with infection and death.**

### **Methods**

**Patients with SV in remission were vaccinated with pneumococcal 7-valent conjugate, Haemophilus influenzae type B and Meningococcal group C conjugate vaccine and Meningococcal polysaccharide groups A, C, Y and W135 vaccines. Total IgG and antibody titres against specific antigens and lymphocyte subset analysis were performed before vaccination. Post vaccination antibody titres were measured at 4 weeks and 2 years from which an antibody response score was calculated. Infections and death following vaccination were collected prospectively following vaccination.**

### **Results**

**92 patients were safely vaccinated with no increase in disease relapse, median follow-up 4.6 (3.6-4.8) years. Eighteen patients died at a median of 2 years and the overall infection rate was 0.4 (0.2-1.3) infections/patient/year. Reduced serum IgG, B cell and CD4+ cell counts predicted poor vaccine response and infection but not death. The response rates to individual vaccine antigens was highly variable, median response rate of 46% (interquartile range 39-58%) of patients**

responding to each individual antigen. Vaccine response, age and reduced renal function were independent predictors of all-cause mortality in multivariate analysis.

## **Conclusion**

**Total IgG and B cell counts predict infection and response to vaccination. Vaccination in patients with SV in remission is safe and the response predicts all-cause mortality. Vaccine response is a surrogate marker of immune system health.**

## **Significance and Innovations:**

- In patients with systemic vasculitis in remission there needs to be a balance of risk between the side-effects associated with immunosuppression and disease activity. This balance must be individualised to each patient but this is often difficult . Measurement of total IgG and B cell counts in patients with systemic vasculitis in remission helps identify those most at risk of infection.
- This study identifies that the humoral response to vaccination predicts those at greatest risk of all-cause mortality, with the commonest cause of death being infection.
- Vaccination in this group of patients is safe
- Humoral response to vaccination, total IgG level and B cell counts taken together may identify a subset of patients who are at greatest risk from the side-effects of immunosuppressive therapy.

Despite improved treatments, patients with systemic vasculitis continue to have increased all-cause mortality compared to healthy individuals. Specifically, patients with ANCA-associated vasculitis have a 2.6 mortality ratio compared with the healthy population, with infection being the most frequent cause of death[1], and associated with prolonged use of immunosuppression. Infection is also an important cause of morbidity. In the first year following diagnosis, when immunosuppression is at its highest, 25% of patients with systemic vasculitis had an infection episode[2]. Although infection risk reduces with time in these patients, it remains elevated compared to the healthy population. The risk of admission to hospital with pneumonia, a vaccine preventable illness, in these patients is increased at up to 5 times that of an age matched healthy population[3]. Identification and prevention of those at high risk of side-effects associated with therapy is essential to improve outcomes in these patients.

Cumulative immunosuppression[4], age and severity of renal impairment [2], are important risk factors for infection and mortality. However no immune biomarkers, beyond leucopenia[5], have been shown to help identify those with poor functional immune systems and increased risk of infection. Vaccination is not only therapeutic but also provides information on the health of the immune system. Vaccination is recommended for immunosuppressed patients to prevent infection; however the immune response is often poorer than in healthy controls[6]. Failure to mount an effective response to vaccination along with detailed investigation of the immune system may identify those patients most at risk of infection and death.

The aim of this study was to investigate whether detailed investigation of the immune system and the immune response to vaccination in patients with systemic vasculitis predicted all-cause mortality and risk of infection.

## METHODS

Patients were identified from the vasculitis clinic lists at University Hospitals Birmingham NHS Foundation Trust (UHBFT). Patients had a diagnosis of small or medium sized systemic vasculitis (granulomatosis with polyangiitis, microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis or classical polyarteritis nodosa) and had not received pneumococcal or haemophilus influenza vaccine within the previous 5 years. Following the introduction of routine vaccination in the clinic in 2009 patients were consented for the use of their clinical data. All patients gave informed consent and the study protocol was approved by the West Midlands Research Ethics Committee (10/H1208/13).

Patients were eligible for vaccination if they had been in stable remission > 6 months (Birmingham Vasculitis Activity Score=0); had received cyclophosphamide and steroid induction therapy but not within 6 months; had not received rituximab within 6 months; were on  $\leq 10$ mg prednisolone per day; currently treated with no more than one immunosuppressant in addition to prednisolone; had no active infection; were not pregnant and had no history of previous severe adverse reaction to vaccination or received vaccination with the proposed vaccines. Clinical and laboratory data were recorded from patient records. Infection episodes following vaccination were prospectively identified from patient notes. Severe infections were defined as requiring admission to hospital and/or intravenous antibiotic therapy. Patients requiring intravenous immunoglobulin (IVIg) were excluded from the study. Causes of death during follow up were determined from death certificate data.

Patients had blood collected immediately pre-vaccination for lymphocyte subset analysis by flow cytometry (CD3, CD4, CD8, CD19, CD16, CD56; all Becton and Dickinson, Oxford, UK) and total immunoglobulin G (IgG) assayed by nephelometry by the Clinical Immunology Laboratory (CIL) at the

University of Birmingham. Vaccinations given were; 0.5ml pneumococcal (Pn) 7-valent conjugate vaccine (Prenar; Pfizer, Surrey, UK ) intra-muscular injection; 0.5ml Haemophilus type b (Hib) and Meningococcal (Men) group C conjugate vaccine (Menitorix) intra-muscular injection at time 0; 0.5ml Meningococcal polysaccharide groups A, C, Y and W<sub>135</sub> vaccine (ACWY Vac) deep subcutaneous injection administered four weeks later (both GlaxoSmithKline, Uxbridge, UK) . Serum was collected immediately pre-vaccination for antibody titres and at 4 weeks and 2 years following each vaccination to assess response.

Antibody titres against Pn, Hib and Men were assayed by the CIL using a multiplex assay which is externally quality assured by United Kingdom National External Quality Assessment Service, Sheffield, UK[7]. Serum was separated from blood and frozen at -20°C until analyzed. There is inherent variability in functional antibody testing so to minimise inter-assay variability samples were batch analysed such that all samples from an individual patient were included in the same analysis. For antibody titre threshold analysis Pn titres >0.35 ug/ml, Men titres >2 ug/ml and Hib >1 ug /ml were considered to be above threshold. It is unknown what a protective pneumococcal antibody titre is in adults and may vary according to serotype and published concentrations vary from 0.15 – 2 ug /ml. A cut off of 0.35 ug /ml is recommended by World Health Organisation for infants and is used widely in the UK for adults.

Antibody response scores were calculated using the formula

$$100 * \frac{(\text{number of antibodies at time } T > \text{threshold} - \text{number of prevaccination antibodies} > \text{threshold})}{(12 - \text{number of antibodies prevaccination} > \text{threshold})}$$

We assessed response as the proportion of below “protective” threshold antibody titres that changed to above threshold titres following vaccination. Where 12 is the total number of antibody titres measured for each patient and T is time post vaccination. Patients with all 12 antibody titres >threshold, could not improve their response from pre-vaccination levels (n=5) and were excluded from this analysis.

## Statistical analysis

Continuous data is presented as median (inter-quartile range) unless stated. Categorical variables are presented as number (percentage). Differences between groups were analysed by Mann-Whitney, Wilcoxon signed rank or Kruskal-Wallis tests for continuous variables or by Pearson's  $\chi^2$  test with Yates' correction for discrete variables as appropriate. Correlations were tested using Spearman's Rank method. Survival analysis was performed using Cox regression and is reported as hazard ratio (HR) and 95% confidence interval (95%CI). Statistical analysis was performed using SPSS version 20 (IBM, Portsmouth, UK). P values <0.05 were considered significant. This was an exploratory observational cohort study and no formal power calculations were included. Our primary analysis was vaccine response with factors predicting that response secondary outcomes.

## RESULTS

Ninety two patients were vaccinated and had clinical and laboratory follow up. The demographic details of the participants are shown in table 1. Median patient follow up post vaccination was 4.6 (3.6-4.8) years and total patient follow up was 363 patient years; none were lost to follow up.

Eighteen patients died during follow up at a median of 2 (0.6-3.0) years post vaccination. Causes of death were infection (n=8), cardiovascular disease (n=3), cancer (n=3) and kidney disease (n=4).

Lymphocyte phenotyping was incomplete in eight patients; their vaccine responses are reported with their clinical details and follow up but they are not included in the analysis of lymphocyte subsets associated with vaccine responses.

Nine patients had previously received rituximab at a median 1.8 (1.2 – 2.6) years prior to vaccination. At the time of vaccination 81 patients were still taking prednisolone at a median of 5 (5-10) mg/day. Seven patients had discontinued immunosuppression prior to vaccination.

During follow up 70 patients experienced 268 infections and 35 patients experienced 56 serious infections (defined as hospital admission and/or iv antibiotics). The commonest infections were



lower respiratory tract (n=116), upper respiratory tract (n=42), urinary tract (n=39), fungal and bacterial skin and mucosal infections (n=30) and gastrointestinal infections (n=16). There were only two reported episodes of herpes zoster infection. Other infections included osteomyelitis (n=2), dialysis related peritonitis (n=1), non-specific viral infections (n=20) and two episodes of bacteremia without an identified source. There were no episodes of pneumocystis jirovecii.

The median infection rate was 0.4 (0.2-1.3) per patient per year for all infections and 0 (0-0.2) serious infections per patient per year following vaccination. The overall infection rate for the cohort was 0.7 infections per year and 0.2 serious infections per year. Vaccination was safe. There were no adverse events reported immediately following vaccination. There was no change in the relapse rate in the two years following vaccination (pre-vaccination 0.15 per patient year; post-vaccination 0.12 per patient year;  $p>0.05$ ).

### **Immune biomarkers predict infection**

At the time of vaccination all patients had a total white cell count within the normal range with normal neutrophil and NK cell counts. The median total lymphocyte count was low, with low CD3<sup>+</sup> T cells and CD19<sup>+</sup> B cells (table 1). Thirty seven percent of patients had CD4<sup>+</sup> cell counts less than the lower limit of normal and 44% patients had CD8<sup>+</sup> cell counts below the normal range. Twenty two percent of patients had low IgG levels. Serum IgG concentration correlated with B cell count ( $cc=0.33$ ;  $p=0.002$ ), and CD4 count ( $cc=0.3$ ;  $p=0.005$ ).

B cell count correlated negatively with previous cumulative cyclophosphamide exposure ( $cc=-0.29$ ;  $p=0.012$ ); CD4 count correlated negatively with age ( $cc=-0.35$ ;  $p=0.001$ ); both B cell count and CD4 T cell count correlated positively with eGFR ( $cc=0.25$ ;  $p=0.022$  and  $cc=0.35$ ;  $p=0.001$  respectively).

Overall infection rates correlated negatively with serum IgG ( $r=-0.27$ ;  $p=0.01$ ), CD4 cell count ( $r=-0.22$ ;  $p=0.049$ ) and B cell count ( $r=-0.22$ ;  $p=0.046$ ) (supplementary figure 1). The serious infection rate correlated negatively with eGFR ( $r=-0.23$ ;  $p=0.028$ ), B cell count ( $r=-0.27$ ;  $p=0.014$ ), and CD4 T

cell count ( $r=-0.25$ ;  $p=0.021$ ).  $CD8^+$  cell counts correlated negatively with age ( $r=-0.27$ ;  $p=0.013$ ) but there was no correlation with the infection or serious infection rates, renal function or cumulative corticosteroid or cyclophosphamide use.

Patients on maintenance immunosuppression at the time of vaccination had lower B cell counts,  $CD4$  T cell counts and serum IgG concentrations but higher eGFR than patients on steroids only or no maintenance therapy (figure 2), suggesting a more dysfunctional immune system. There was no difference in  $CD8^+$  counts between those still on maintenance immunosuppression and those who had discontinued it.

Patients on maintenance immunosuppression had more infections per year during follow up (0 (0-0.2) vs 0.6 (0.2-1.5);  $p=0.0001$ ) although there was no difference in the serious infection rate between patients on maintenance immunosuppression and those who had discontinued immunosuppression (0 (0-0.2) vs 0 (0-0.2);  $p=0.7$ ). Previous cumulative steroid dose correlated with the overall infection rate ( $r=0.21$ ;  $p=0.043$ ) but not the serious infection rate ( $r=0.18$ ;  $p=0.097$ ).

There was no difference between patients still on immunosuppression and those who had discontinued with regards to age and previous cyclophosphamide or steroid use. There was no relationship between previous cumulative cyclophosphamide use and the infection or serious infection rate. There was no correlation between age and all infections but there was a non-significant correlation between age and the serious infection rate ( $r=0.2$ ;  $p=0.056$ ).

### **Vaccine Response**

The median antibody titres for all the vaccine components increased at 4 weeks post vaccination (supplementary table 1) whereas there was no significant change in the antibody titres against Pn1, Pn3, Pn5 and Pn7 which were not included in the 7-valent conjugate vaccine. At four weeks post vaccination there was a significant improvement in the percentage of patients who had antibody

titres above the threshold, although there was variability in the response between antigens (antibody response above the “protective” threshold for each antigen median 46% (interquartile range 39-58%)(table 2).

Serum was available for antibody assay from 64 patients two years after vaccination. Although there was a statistically significant reduction in the antibody titres for some antigens after two years (Pn4, Pn6B, Pn9V, Pn18C, Pn23F, MenC & MenY; supplementary table 2) There was no change in the percentages of patients with protective antibody titres against any antigen except for MenC (38% vs 20%;  $p=0.032$ ).

### **Better vaccine responses are associated with a more “intact” immune system**

Patients with higher CD4<sup>+</sup> and CD19<sup>+</sup> cell counts and higher serum IgG had better responses to vaccination. Vaccine response scores correlated with serum IgG concentration ( $r=0.34$ ;  $p=0.001$ ), B cell count ( $r=0.36$ ;  $p<0.001$ ) and CD4 cell count ( $r=0.24$ ;  $p=0.028$ )(supplementary figure 1).

Patients on no immunosuppression had better vaccine response scores than patients still on maintenance immunosuppression (75 (25-88)% vs 50 (17-60)%;  $p=0.042$ ). The response score did not correlate with patient age, cumulative cyclophosphamide or steroids or eGFR. There was no correlation between the total infection rate or the serious infection rate and vaccine response scores.

### **Poor vaccine response is associated with a higher risk of all-cause mortality**

The median vaccine response score was 46%. Patients were classified into those with a vaccine response score >50% or <50%. Kaplan-Meier survival analysis demonstrated that patients with vaccine response scores >50% had better survival than those with vaccine response scores less than 50% (figure 1).

In Cox regression multivariable survival analysis increased age, poor renal function and reduced response to vaccination were significantly and independently associated with increased risk of all-cause mortality (table 3). In this cohort of patients in established stable remission the overall and serious infection rates post vaccination predicted survival in the univariable survival analysis (supplementary table 4) but not the multivariable analysis. Patients who subsequently died from infection had significantly worse vaccine response scores than patients who were alive at the end of follow up (29% (5-42%) vs 50% (25-74%);  $p=0.044$ ) although this was not significantly different to patients who died from other causes (33% (20-40%);  $p=0.8$ ).

### **DISCUSSION**

All-cause mortality and infection remains increased in patients with systemic vasculitis compared to the general population [1, 2, 8]. This study identifies that low IgG and low B cells, immunological biomarkers, are associated with infection and vaccine response in patients with systemic vasculitis in stable remission on maintenance immunosuppression. Low CD4 counts were associated with severe infection. This study confirms the association of poor renal function and continued immunosuppression with increased risk of infection. Although a poor response to vaccination was not associated with infection risk it was identified as an important predictor of all-cause death in patients with systemic vasculitis, independent of age and renal function.

Surprisingly, despite patients being in stable remission for at least 6 months from the time of last receiving cyclophosphamide, a large percentage remained lymphopenic, particularly with pronounced B cell lymphopenia. The negative correlation of B cell counts with prior cyclophosphamide exposure suggests that the effects of cyclophosphamide on the immune system are long lasting. The association of lymphopenia and low CD4+ counts with immune dysfunction and infection has been reported but this study extends understanding by clearly showing the association of low B cell counts with infection and poor vaccine response in this population.

Only 9 of our patients had received rituximab and all at least 6 months prior to vaccination. It is well known that rituximab reduces the efficacy of vaccination, especially if given within the first few months post rituximab treatment [9]. This study suggests that B cell depletion due to other causes, such as cyclophosphamide, also influence the efficacy of vaccination, along with low IgG levels and CD4+ cell counts. We have also identified effects of anti-proliferative immunosuppressants on vaccine responses. This differs to previous GPA patients vaccinated with influenza, which showed no effect of immunosuppressive therapy[10]. The effect seen in our study may be due to differences in vaccines used and the larger cohort size in our study.

The response of patients to individual antigens was poor with fewer than 50% of patients responding to most antigens. A poor response to the vaccination was indicative of poor immunological function with low IgG, B cell counts and CD4+ T cell counts predicting vaccine response score. Those patients with a poor vaccine response were more likely to be on maintenance immunosuppression. This study extends previous findings that identified low CD4+ T cell counts as a risk for lack of humoral immunity to tetanus[11]. B cell counts have been identified as predictive of a memory response to vaccination in children with HIV[12]. Although other studies have shown reduced response to vaccination in immunosuppressed populations, this is the first study to assess immunological predictors of vaccine response in an adult population with systemic vasculitis on immunosuppressive therapy.

Previously there has been concern regarding the risk of vaccination triggering a relapse of vasculitis. We found no increase in the risk of relapse following vaccination. Similar findings have been reported in previous studies of influenza vaccination in AAV patients[10, 13-15].

We used conjugate pneumococcal vaccine in this study, as a rapid decrease in antibody levels to the polysaccharide vaccine, pneumovax, has been reported in elderly and immunocompromised patients[16-18]. In our study patients able to mount an effective immune response maintained “protective” antibody responses up to 2 years, suggesting that vaccination remains an important preventative measure against infection. However absolute titres of antibody did decline over the period of follow up. Similar results were found in patients with inflammatory arthritis who showed a reduction in antibody titres over 18 months[19]. Both these studies suggest that in patients who are immunocompromised early revaccination strategies may be required.

Although vaccination response did not predict infection, those cellular and humoral biomarkers predicting poor vaccine response did predict infection in patients with systemic vasculitis in stable remission. Low serum IgG, B cell counts, cumulative steroid exposure and maintenance therapy were identified as important risk factors for any infection, but not all-cause mortality, while reduced renal function and CD4+ T cell counts additionally increased the risk for severe infection. This study suggests the vaccination score provides useful information on the health of the immune system and provides a surrogate marker of poor immune response, which may identify those patients potentially benefitting from a reduction in immunosuppression. For those who require long-term immunosuppression due to continued disease activity, this data may help identify those patients who require additional intervention to reduce risk of infection.

This study confirmed previous associations between infection and impaired renal function, corticosteroid exposure and lymphopenia[20, 21] [22]. We extended these observations by demonstrating that CD4 cell counts were associated with severe infection and reduced B cells and

low serum IgG was associated with an increased risk of all infections. Our study differs from previous reports[4, 20, 23], in that we did not find a direct association between previous cyclophosphamide use and infection. This may reflect the reduced cumulative dose when using pulsed cyclophosphamide regimens, which is associated with fewer episodes of leucopenia compared with daily oral cyclophosphamide[24]. We did, however, show a correlation between cyclophosphamide exposure and B cell count suggesting that, even in an era of reduced cyclophosphamide use the long term impact on the immune system may be important.

In our study patients at risk of all-cause mortality as predicted by a poor vaccination response have a combined deficiency in both the cellular and humoral parts of the immune system. Severe infection rates were similar to that seen in primary immunodeficiencies, such as combined variable immunodeficiency syndrome (CVID). Immune deficiencies in patients with CVID are characterised by a reduced serum IgG, and reduced subsets of B cells and T cells in the peripheral blood[25]. There is an increased risk of susceptibility to respiratory infection with encapsulated bacteria[26]. Patients with CVID treated with IVIg reconstitution have reduced infection and improved cellular immunity[27]. The commonest infection described in patients with systemic vasculitis is respiratory tract infection [3]. IVIg replacement may be beneficial for patients with systemic vasculitis and recurrent infection, who have low IgG levels, reduced CD4+ and CD19+ lymphocytes and respond poorly to vaccination.

Several limitations of this study require comment. Information on the duration of pneumocystis jiroveci prophylaxis was not available in sufficient detail to allow comment on its ability to reduce bacterial infection in this cohort. Although vaccination aims to reduce infection, due to the large sample size required for such a study and the difficulty in proving bacterial diagnosis this study was not powered to investigate changes in infection rates in response to vaccination. Good data exists for predictors of infection and mortality and vaccine responses are known to be lower during times of intense immunosuppression. We, therefore, focussed on patients who were in sustained

remission and had not received induction therapy within the last 6 months and followed them prospectively, as little has previously been known about risk factors for infection or all-cause mortality in this group. Infection rates in this study are lower than previously reported and reflect the reduced immunosuppression used in patients in stable remission and may explain the lack of association between vaccination response and infection. We included meningitis vaccination in this study, although not standard vaccination for this group, it allowed us to assess both T cell dependent and T independent vaccines. As expected response to the polysaccharide vaccinations were lower than to the conjugate vaccines but all patients did respond to a degree. Although this may limit the applicability of the vaccine response score, it provided important information on the response to polysaccharide vaccines.

In conclusion, this study identifies older patients with poor renal function and vaccine responses to be at highest risk of all-cause mortality. In addition, those with low B cells and IgG levels had greatest risk of infection. Using these biomarkers as risk stratification may allow identification of patients who may benefit from immunosuppression withdrawal if appropriate and/or the use of additional therapies, such as IVIg, as a way to reduce infection and mortality.

## **Disclosure**

Dr. Goldblatt reports grants from Vaccine manufacturers: GSK, Sanofi Pasteur, Merck, Novartis, personal fees from Vaccine manufacturers: GSK, Sanofi Pasteur, Merck, Novartis, Pfizer, outside the submitted work.

Dr Alex Richter reports grant funding from Pfizer outside the submitted work

None of the other authors has relevant potential conflicts of interest to disclose.

## **ACKNOWLEDGEMENTS**



The work was carried out at the National Institute for Health Research (NIHR)/Wellcome Trust Birmingham Clinical Research Facility. The views expressed are those of the authors(s) and not necessarily those of the NHS, the NIHR or the Department of Health. All authors have contributed to the study design, data collection, analyses and drafting of the manuscript and agreement of the final version for publication. We would like to thank Dr P Nightingale, statistician NIHR/Wellcome Trust Birmingham Clinical Research Facility, UHB NHS Foundation Trust, for his statistical advice.

No financial support was received for this work.

## REFERENCES

1. Flossmann O, Berden A, de Groot K, Hagen C, Harper L, Heijl C, Hoglund P, Jayne D, Luqmani R, Mahr A *et al*: **Long-term patient survival in ANCA-associated vasculitis**. *Ann Rheum Dis* 2011, **70**(3):488-494.
2. Little MA, Nightingale P, Verburgh CA, Hauser T, De Groot K, Savage C, Jayne DR, Harper L: **Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis**. *Ann Rheum Dis* 2010, **69**:1036-1043.
3. Wotton CJ, Goldacre MJ: **Risk of invasive pneumococcal disease in people admitted to hospital with selected immune-mediated diseases: record linkage cohort analyses**. *Journal of epidemiology and community health*, **66**(12):1177-1181.
4. Charlier C, Henegar C, Launay O, Pagnoux C, Berezne A, Bienvenu B, Cohen P, Mouthon L, Guillemin L: **Risk factors for major infections in Wegener granulomatosis: analysis of 113 patients**. *Ann Rheum Dis* 2009, **68**(5):658-663.
5. Wall N, Harper L: **Complications of long-term therapy for ANCA-associated systemic vasculitis**. *Nat Rev Nephrol* 2012, **8**(9):523-532.
6. Saad CG, Borba EF, Aikawa NE, Silva CA, Pereira RM, Calich AL, Moraes JC, Ribeiro AC, Viana VS, Pasoto SG *et al*: **Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases**. *Ann Rheum Dis* 2011, **70**(6):1068-1073.
7. Whitelegg AM, Birtwistle J, Richter A, Campbell JP, Turner JE, Ahmed TM, Giles LJ, Fellows M, Plant T, Ferraro AJ *et al*: **Measurement of antibodies to pneumococcal, meningococcal and haemophilus polysaccharides, and tetanus and diphtheria toxoids using a 19-plexed assay**. *J Immunol Methods* 2012, **377**(1-2):37-46.

8. Mukhtyar C, Flossmann O, Hellmich B, Bacon P, Cid M, Cohen-Tervaert JW, Gross WL, Guillevin L, Jayne D, Mahr A *et al*: **Outcomes from studies of antineutrophil cytoplasm antibody associated vasculitis: a systematic review by the European League Against Rheumatism systemic vasculitis task force.** *Ann Rheum Dis* 2008, **67**(7):1004-1010. Epub 2007 Oct 1002.
9. Hua C, Barnetche T, Combe B, Morel J: **Effect of methotrexate, anti-tumor necrosis factor alpha, and rituximab on the immune response to influenza and pneumococcal vaccines in patients with rheumatoid arthritis: a systematic review and meta-analysis.** *Arthritis care & research* 2014, **66**(7):1016-1026.
10. Holvast A SC, Benne CA, Huckriede A, Wilschut JC, Palache AM, Kallenberg CG, Bijl M: **Wegener's granulomatosis patients show an adequate antibody response to influenza vaccination.** *Ann Rheum Dis* 2009, **68**(6):873-878.
11. Marchand-Janssen C, Loulergue P, Mouthon L, Mahr A, Blanche P, Deforges L, Lebon P, Cohen P, Pagnoux C, Le Guern V *et al*: **Patients with systemic inflammatory and autoimmune diseases are at risk of vaccine-preventable illnesses.** *Rheumatology (Oxford)* 2011, **50**(6):1099-1105.
12. Abzug MJ, Song LY, Levin MJ, Nachman SA, Borkowsky W, Pelton SI: **Antibody persistence and immunologic memory after sequential pneumococcal conjugate and polysaccharide vaccination in HIV-infected children on highly active antiretroviral therapy.** *Vaccine* 2013, **31**(42):4782-4790.
13. Kostianovsky A, Charles P, Alves JF, Goulet M, Pagnoux C, Le Guern V, Mouthon L, Krivine A, Villiger P, Launay O *et al*: **Immunogenicity and safety of seasonal and 2009 pandemic A/H1N1 influenza vaccines for patients with autoimmune diseases: a prospective, monocentre trial on 199 patients.** *Clin Exp Rheumatol* 2012, **30**(1 Suppl 70):S83-89.
14. Stassen PM, Sanders JS, Kallenberg CG, Stegeman CA: **Influenza vaccination does not result in an increase in relapses in patients with ANCA-associated vasculitis.** *Nephrol Dial Transplant* 2008, **23**(2):654-658. Epub 2007 Oct 2002.
15. Zycinska K, Romanowska M, Nowak I, Rybicka K, Wardyn KA, Brydak LB: **Antibody response to inactivated subunit influenza vaccine in patients with Wegener's granulomatosis.** *J Physiol Pharmacol* 2007, **58 Suppl 5**(Pt 2):819-828.
16. Hedlund J, Ortqvist A, Konradsen HB, Kalin M: **Recurrence of pneumonia in relation to the antibody response after pneumococcal vaccination in middle-aged and elderly adults.** *Scandinavian journal of infectious diseases* 2000, **32**(3):281-286.
17. Sankilampi U, Honkanen PO, Bloigu A, Leinonen M: **Persistence of antibodies to pneumococcal capsular polysaccharide vaccine in the elderly.** *The Journal of infectious diseases* 1997, **176**(4):1100-1104.
18. Kumar D, Welsh B, Siegal D, Chen MH, Humar A: **Immunogenicity of pneumococcal vaccine in renal transplant recipients--three year follow-up of a randomized trial.** *Am J Transplant* 2007, **7**(3):633-638.
19. Crnkic Kapetanovic M, Saxne T, Truedsson L, Geborek P: **Persistence of antibody response 1.5 years after vaccination using 7-valent pneumococcal conjugate vaccine in patients with arthritis treated with different antirheumatic drugs.** *Arthritis Res Ther* 2013, **15**(1):R1.
20. Goupil R, Brachemi S, Nadeau-Fredette AC, Deziel C, Troyanov Y, Lavergne V, Troyanov S: **Lymphopenia and treatment-related infectious complications in ANCA-associated vasculitis.** *Clin J Am Soc Nephrol* 2013, **8**(3):416-423.
21. McGregor JG, Hogan SL, Hu Y, Jennette CE, Falk RJ, Nachman PH: **Glucocorticoids and relapse and infection rates in anti-neutrophil cytoplasmic antibody disease.** *Clin J Am Soc Nephrol* 2012, **7**(2):240-247.
22. Weidanz F, Day CJ, Hewins P, Savage CO, Harper L: **Recurrences and infections during continuous immunosuppressive therapy after beginning dialysis in ANCA-associated vasculitis.** *Am J Kidney Dis* 2007, **50**(1):36-46.

23. Booth AD, Almond MK, Burns A, Ellis P, Gaskin G, Neild GH, Plaisance M, Pusey CD, Jayne DR: **Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study.** *Am J Kidney Dis* 2003, **41**(4):776-784.
24. de Groot K, Harper L, Jayne D, Flores Suarez L, Gregorini G, Gross W, al e , Group). EEVS: **Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody--associated vasculitis. A randomized trial.** . *Ann Intern Med* 2009, **150**:670-680.
25. Moratto D, Gulino AV, Fontana S, Mori L, Pirovano S, Soresina A, Meini A, Imberti L, Notarangelo LD, Plebani A *et al*: **Combined decrease of defined B and T cell subsets in a group of common variable immunodeficiency patients.** *Clin Immunol* 2006, **121**(2):203-214.
26. Goldacker S, Draeger R, Warnatz K, Huzly D, Salzer U, Thiel J, Eibel H, Schlesier M, Peter HH: **Active vaccination in patients with common variable immunodeficiency (CVID).** *Clin Immunol* 2007, **124**(3):294-303.
27. Paquin-Proulx D, Santos BA, Carvalho KI, Toledo-Barros M, Barreto de Oliveira AK, Kokron CM, Kalil J, Moll M, Kallas EG, Sandberg JK: **IVIg immune reconstitution treatment alleviates the state of persistent immune activation and suppressed CD4 T cell counts in CVID.** *PLoS One* 2013, **8**(10):e75199.

**Table 1**

Median (IQR) or N=	Patients who received vaccination N=92
Age (years)	66 (53-74)
Male:Female (n:n)	52:40
Time since diagnosis (years)	5.7 (2.6-8.7)
eGFR (ml/min/1.73m <sup>2</sup> )	62 (38-78)
Cumulative cyclophosphamide (g)	10 (5-18)
Cumulative prednisolone (g)	14.2 (8.9-22.1)
Median dose prednisolone	5 (5-10)
N treated with rituximab	9
N treated with immunosuppressants	35 azathioprine 34 mycophenolate 17 nil
GPA:MPA:EGPA:PAN (n:n:n:n)	59:22:7:4
Lymphocyte count mm <sup>3</sup> (IQR) [normal range]	882 (560 – 1370) [1000-4000]
CD3 count/mm <sup>3</sup> (IQR) [normal range]	675 (385 – 1033) [700-1200]
CD4 count/mm <sup>3</sup> (IQR) [normal range]	373 (229 – 581) [300-1400]
CD8 count /mm <sup>3</sup>	244 (144 – 425) [200-900]
CD19 B cell count mm <sup>3</sup> (IQR) [normal range]	30 (10 – 110) [100-500]
Serum IgG g/L (IQR) [normal range]	8.5 (6.4-10.7) [5.4-16.1]

Demographic and therapy and laboratory details of the patients at the time of vaccination. The normal ranges reported by the Clinical Immunology Laboratory for peripheral blood lymphocyte, CD3, CD4, CD8 and CD19 cell counts and serum IgG are shown in square brackets. Data is shown as median (interquartile range).

Abbreviations: N, number of patients; IQR, interquartile range; eGFR estimated glomerular filtration rate using the modified MDRD formula; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; PAN, classical polyarteriitis nodosa, IgG, immunoglobulin

**Table 2**

Serotype	Percentage of patients with antibody titre > threshold		p=
	Pre-vaccination	Post-vaccination	
Pn4	23%	42%	<0.001
Pn6B	48%	67%	<0.001
Pn9V	55%	82%	<0.001
Pn14	55%	74%	<0.001
Pn18C	70%	88%	<0.001
Pn19F	59%	77%	<0.001
Pn23F	50%	74%	<0.001
Men A	33%	79%	0.029
Men C	9%	54%	0.006
Men W135	2%	23%	0.4
Men Y	12%	49%	0.001
Hlb	26%	68%	0.001

The percentage of patients with antibody titres above the protective threshold pre-vaccination and four weeks post vaccination.

Abbreviations: Pn, pneumococcal serotype, Men, meningitis serotype, Hlb, Haemophilus Influenza b

**Table 3**

Factor	Hazard ratio (95% CI)	P
Age (per 10 years)	2.46 (1.12 – 5.38)	0.024
eGFR (per 10ml/minute)	0.21 (0.08 – 0.57)	0.002
Vaccine response score (<50% response)	6.01 (1.67 – 21.67)	0.006

Multivariable Cox regression survival analysis of factors associated with mortality.

Abbreviations: CI, confidence intervals; eGFR, estimated glomerular filtration rate using the modified MDRD formula

Supplementary table 1

Serotype	Week 0 titre	Week 4 titre	p=
Pn4	0.1 (0.1 - 0.3)	0.2 (0.1 - 0.9)	<0.001
Pn6B	0.3 (0.1 - 1.3)	0.9 (0.3 - 6.1)	<0.001
Pn9V	0.5 (0.1 - 1.4)	1.8 (0.6 - 10)	<0.001
Pn14	0.5 (0.1 - 1.9)	1.9 (0.3 - 10)	<0.001
Pn18C	1.0 (0.2 - 3.5)	4 (1.1 - 10)	<0.001
Pn19F	0.5 (0.2 - 1.2)	1.1 (0.4 - 3.2)	<0.001
Pn23F	0.4 (0.1 - 1.9)	2.5 (0.4 - 9.6)	<0.001
Men A	1.2 (0.6 - 2.3)	4 (1.9 - 9.2)	<0.001
Men C	0.1 (0 - 0.2)	1.7 (0.5 - 8.1)	<0.001
Men W135	0 (0 - 0.1)	0.2 (0 - 1.6)	<0.001
Men Y	0.2 (0.1 - 0.6)	2.4 (0.4 - 20)	<0.001
HIb	0.2 (0 - 1.1)	2.3 (0.5 - 20)	<0.001

The antibody titres for the serotypes included in the vaccination regime at week 0 (pre-vaccination) and 4 weeks following vaccination. P values are for paired tests comparing week 0 and week 4

Abbreviation Pn, pneumococcal; Men, meningococcal; HIb, Haemophilous Influenza b



Supplementary table 2

	<b>Percentage of patients with antibody titre &gt; threshold at two years post vaccination</b>		
<b>Serotype</b>	<b>Patients with antibody titres &gt;threshold prevaccination</b>	<b>Patients with antibody titres &lt;threshold pre-vaccination who responded to vaccination</b>	<b>P for <math>\chi^2</math> Yates' correction</b>
Pn4	80%	62%	ns
Pn6B	94%	75%	ns
Pn9V	96%	72%	0.023
Pn14	98%	100%	ns
Pn18C	100%	93%	ns
Pn19F	94%	57%	0.009
Pn23F	85%	70%	ns
Men A	98%	66%	0.002 (0.167)
MenC	100%	70%	0.031 (0.079)
Men W135	100%	88%	ns
Men Y	94%	82%	ns
Hib	95%	100%	ns

The percentage of patients with antibody titre above the relevant threshold at two years for patients with pre-existing antibodies pre-vaccination and patients without pre-existing antibodies but who responded to vaccination demonstrating above threshold titres at 4 weeks post vaccination.

Abbreviation Pn, pneumococcal; Men, meningococcal; Hib, Haemophilous Influenza b

Supplementary Table 3:

Serotype	Baseline	4 weeks	2 years	P (baseline vs 4 weeks)	P (4 weeks vs 2 years)
Pn4	0.04 (0.02-0.12)	0.10 (0.05-0.44)	0.10 (0.04-0.30)	<0.001	0.006
Pn6B	0.09 (0.03-0.34)	0.22 (0.07-1.62)	0.22 (0.08-1.01)	<0.001	0.009
Pn9V	0.23 (0.11-0.59)	0.82 (0.25-2.99)	0.74 (0.22-2.05)	<0.001	<0.001
Pn14	0.84 (0.35-3.92)	1.25 (0.61-3.93)	1.17 (0.57-4.31)	<0.001	ns
Pn18C	0.39 (0.15-0.90)	1.31 (0.35-8.23)	1.14 (0.39-4.29)	<0.001	0.029
Pn19F	0.36 (0.15-1.29)	0.54 (0.21-1.57)	0.54 (0.21-2.27)	<0.001	ns
Pn23F	0.37 (0.12-1.19)	1.65 (0.26-4.51)	0.87 (0.23-4.46)	<0.001	0.002
MenA	2.10 (0.9-3.57)	4.32 (2.37-10.00)	4.81 (2.60-10.00)	<0.001	ns
MenC	0.08 (0.04-0.22)	1.03 (0.23-4.27)	0.45 (0.16-1.82)	<0.001	<0.001
MenW135	0.14 (0.08-0.34)	0.36 (0.14-2.41)	0.41 (0.15-1.77)	<0.001	ns
MenY	0.19 (0.09-0.41)	0.66 (0.19-3.88)	0.58 (0.17-1.86)	<0.001	0.03
Hib	1.01 (0.57-2.57)	2.47 (1.19-9.14)	2.69 (1.14-8.01)	<0.001	ns

The antibody titres for the serotypes included in the vaccination schedule at baseline, 4 weeks and 2 years for the 64 patients who had follow up available at 2 years. P values are for paired sample analysis (Wilcoxon signed rank test) comparing baseline vs 4 weeks and 4 weeks vs 2 years. ns indicates  $p > 0.05$ .

Abbreviation Pn, pneumococcal; Men, meningococcal; Hib, Haemophilous Influenza b

Supplementary Table 4

Factor	Hazard ratio (95% CI)	P
Age (per 10 years)	2.2 (1.5 - 3.3)	<0.001
eGFR (per 10ml/minute/1.73m <sup>2</sup> )	0.65 (0.53 – 0.8)	<0.001
Vaccine response score (per 10%)	0.87 (0.76 – 0.995)	0.042
Severe infection rate per year	5 (1.7 - 14.8)	0.003
Infection rate per year	1.6 (1.02 – 2.4)	0.039
Previous cumulative CYC (per g)	0.97 (0.91 – 1.0)	0.277
Previous cumulative steroids (per g)	1 (0.96-1.04)	0.979
Continued maintenance immunosuppression	1.7 (0.6 – 4.8)	0.314
Serum IgG (per g)	1.0 (0.9 – 1.2)	0.633
B cell count (per 50 cells)	0.8 (0.6 – 1.1)	0.2
CD4 <sup>+</sup> count (per 50 cells)	0.9 (0.8 – 1.0)	0.063

Univariable analysis of factors associated with increased risk of mortality during follow up.

Abbreviations: eGFR, estimated glomerular filtration rate using the modified MDRD formula; CYC, cyclophosphamide; IgG, immunoglobulin; g, gramme

## FIGURE LEGENDS

Figure 1: Kaplan-Meier survival analysis of patients following vaccination stratified by vaccine response score. Patients with the highest vaccine response scores (>50%) had significantly better survival than patients with lower vaccine response scores ( $p=0.025$ ).

Figure 2 : The effect of continued azathioprine ( $n=35$ ), mycophenolate (34) or no additional immunosuppression ( $n=17$ ) on MDRD eGFR (A), serum IgG concentration (B), CD19 B cell count (C) and CD4 T cell count (D). Patients on no additional immunosuppression had lower eGFR and higher serum IgG, B and T cell counts.

Supplementary Figure 1: The relationships between infection rates per year and serum immunoglobulin G (A), CD4 T cells (B) and CD19 B cells (C).

Supplementary Figure 2: The relationships between vaccine response scores and serum immunoglobulin G (A), CD19 B cells (B), CD4 T cells (C) and infection rates per year (D).